PATENT
Attorney Docket No. **OPHD-03282**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	John A. Kink, et al.	
Serial No.:	09/095,536	Art Unit: 1646
Filed:	6/10/98	Examiner: HISSONG, BRUCE D
Entitled:	**Prevention and Treatment of Sepsis**	

APPEAL BRIEF
APPEAL NO.:

ATTENTION: Board of Patent Appeals and Interferences
Mail Stop - Appeals
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CERTIFICATE OF ELECTRONIC FILING

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being deposited with the U.S. Patent and Trademark Office, via EFS.

Dated: June 30, 2008 By: Traci E. Light

BPAI:

This Brief is submitted following a Notice of Appeal filed April 30, 2008. The Brief is therefore believed to be timely and thus no extensions of time are needed.

This Brief is transmitted as a single copy as per the amended rules. [37 CFR § 41.37(a).]

This Brief contains these items under the following headings and in the order set forth below [37 CFR § 1.192(c)]

I. REAL PARTY IN INTEREST 3
II. RELATED APPEALS AND INTERFERENCES 3
III. STATUS OF CLAIMS 3
IV. STATUS OF AMENDMENTS 3
V. SUMMARY OF CLAIMED SUBJECT MATTER 3
VI. GROUNDS OF REJECTION TO BE REVIEWED UPON APPEAL 4
VII. ARGUMENT 5
A. Skurkovich Is Non-analogous Art 5
B. The Examiner's Assertions About "Symptoms" Are Wrong 6
C. The Examiner Overstates What Skurkovich Teaches 7
D. The Examiner Ignores Skepticism And Teaching Away By Experts 7
E. The Examiner Provides Only Conclusory Statements 8
F. Doherty Administered *Prior* To Challenge Using *Single* Antibodies 9
G. Starnes Administered *Prior* To Challenge Using *Single* Antibodies 9
H. Starnes Only Simultaneous Administration Failed 9
I. Applicants Presented Unexpected Results 10
J. The Examiner Acknowledged The 132 Declaration But Did Not Address All Of The Evidence Therein 11
K. The Examiner's Arguments Are Arbitrary And Based On Unfounded Speculation 12
L. Conclusion 12
VIII. CLAIMS APPENDIX i
IX. EVIDENCE APPENDIX – 132 Declaration (of record and Opal *et al.*) iii
X. RELATED PROCEEDINGS APPENDIX iv

I. REAL PARTY IN INTEREST

The real party in interest is Promega Corporation, Madison, WI, assignee of the technology.

II. RELATED APPEALS AND INTERFERENCES

There are no related applications pending appeal.

III. STATUS OF CLAIMS

Claims 49-57 are pending and stand rejected. Claims 1-48 are canceled.

IV. STATUS OF AMENDMENTS

All amendments in the case have been entered.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The present invention contemplates treatment of mammals having symptoms of a systemic septic reaction (pg 4, lines 2-3). In one embodiment (Claim 49), the present invention contemplates a method of treatment, comprising: a) providing i) a mammal having a plurality of symptoms of sepsis, wherein said symptoms comprise arterial hypotension and at least one selected from the group consisting of metabolic acidosis, fever, decreased systemic vascular resistance, tachypnea, and organ failure (pg 3, lines 9-10), ii) a therapeutic preparation, comprising anti-TNF-alpha, anti-IL-6, and anti-IFN antibodies (pg 16, lines 1-12); and b) administering said preparation to said mammal wherein said symptoms are reduced (pg 5, line 14) (Table 5). In one embodiment (Claim 50), said antibodies are polyclonal (pg 4, lines 17).

The present invention also contemplates, in one embodiment (Claim 51), a therapeutic composition for use with a mammal having a plurality of symptoms of sepsis, wherein said symptoms comprise arterial hypotension and at least one selected from the group consisting of metabolic acidosis, fever, decreased systemic vascular resistance, tachypnea, and organ failure (pg 3, lines 9-10), said therapeutic composition comprising avian (pg 4, lines 13-14) anti-TNF-alpha, anti-IL-6, and anti-IFN antibodies (pg 16, lines 1-12) (Table 5).

The present invention also contemplates, in one embodiment (Claim 52), A method of treatment, comprising: a) providing: i) a mammal having sepsis, ii) a therapeutic preparation,

comprising anti-TNF-alpha, anti-IL-6, and anti-IFN antibodies (pg 16, lines 1-12) (Table 5); and b) administering said preparation to said mammal wherein said sepsis is reduced (pg 5, line 14) (Table 5). In one embodiment (Claim 53), said antibodies are polyclonal (pg 4, lines 17).

The present invention also contemplates, in one embodiment (Claim 54), a therapeutic composition for use with a mammal having sepsis, said therapeutic composition comprising avian (pg 4, lines 13-14) anti-TNF-alpha, anti-IL-6, and anti-IFN antibodies (pg 16, lines 1-12) (Table 5).

The present invention also contemplates, in one embodiment (Claim 55), a method of treatment, comprising: a) providing: i) a mammal having septic shock (pg 4, lines 19-21), ii) a therapeutic preparation, comprising anti-TNF-alpha, anti-IL-6, and anti-IFN antibodies (pg 16, lines 1-12) (Table 5); and b) administering said preparation to said mammal wherein said septic shock is reduced (pg 5, line 14) (Table 5). In one embodiment (Claim 56), said antibodies are polyclonal (pg 4, lines 17).

The present invention also contemplates, in one embodiment (Claim 57), a therapeutic composition for use with a mammal having septic shock (pg 4, lines 19-21), said therapeutic composition comprising avian anti-TNF-alpha, anti-IL-6, and anti-IFN antibodies (pg 16, lines 1-12) (Table 5).

VI. GROUNDS OF REJECTION TO BE REVIEWED UPON APPEAL

A. Whether Claims 49-57 are properly rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable in view of Skurkovich taken together with Doherty and Starnes.

B. Whether Applicants Presented Unexpected Results

C. Whether The Examiner Acknowledged The 132 Declaration But Did Not Address All Of The Evidence Therein

D. Whether The Examiner's Arguments Are Arbitrary And Based On Unfounded Speculation

VII ARGUMENT

The Examiner has in a Final Office Action rejected Claims 49-57 under 35 U.S.C. Section 103(a) as allegedly being unpatentable over Skurkovich et al. (US 5,888,511) in view of Starnes et al. (*J. Immunol.*, 1990, vol. 145, pp. 4185-4191) and further in view of Doherty et al. *J. Immunol.*, 1990, vol. 149, pp. 1666-1670). Applicant appeals and asks the BPAI to take note of the fact that this application has been in prosecution ten (10) years with three (3) different examiners. Applicants attempted to appeal the case in January of 2005, only to have the prosecution re-opened in July of 2005 by the previous examiner. In September of 2006, the current examiner took over and now cites a secondary reference (Doherty) that was originally asserted (and later dropped) by the first examiner in 1999. It is also respectfully submitted that the current Examiner has adopted an arbitrary position based on unfounded speculation, without giving due weight to Applicants' evidence of unexpected results. In sum, it is submitted that the treatment of this application by the Patent Office is contrary to the rules of examination (because of piecemeal examination and revisiting prior art dropped long ago) and the law concerning obviousness (which demands that the examiners consider ALL of the evidence).

A. Skurkovich is non-analogous art.

The Examiner uses Skurkovich as the primary reference. However, Skurkovich is directed to the treatment of autoimmune disease, not to sepsis. The Federal Circuit has outlined a basic definition for non-analogous art:

> The determination whether prior art is analogous involves some factual issues concerning whether the reference is within the field of the inventor's endeavor or reasonably pertinent to the particular problem with which the invention was involved.

Finish Engineering Co., Inc. v. Zerpa Industries, Inc., 806 F.2d 1041, 1 USPQ2d 1114, 1116 (Fed. Cir. 1986). Treating autoimmune disease is not "within the field of the inventor's endeavor". The problem Applicant seeks to solve is the morbidity and mortality associated with sepsis. Thus, Skurkovich is not "reasonable pertinent to the particular problem to which the invention was involved". The Examiner is reminded that the determination of whether an inventor would consider a specific reference is based upon the expressed purpose of the reference:

> If a reference disclosure has the same purpose as the claimed invention, the reference relates to the same problem ... If it is directed to a different purpose, the inventor would accordingly have had less motivation or occasion to consider it.

Since Skurkovich has a different purpose, there is no reason for one skilled in the art to consider it. The Examiner has offered no reasons why one seeking to treat sepsis would even look at Skurkovich. In this regard, Appellants ask the Board to consider the central problem of sepsis outlined in the present specification:

> "Many patients with septicemia or suspected septicemia exhibit a rapid decline over a 24-48 hour period." (pg 2, lines 13-14)

This problem associated with sepsis is unlike other diseases (e.g autoimmunity, AIDS) where the progression of disease is over years – not hours. Thus, Skurkovich is irrelevant.

B. The Examiner's Assertions About "Symptoms" Are Wrong

In the Final Office Action (at page 3), in order to justify the use of Skurkovich, the Examiner – for the first time – argues that at least one of Applicants claims are NOT directed to treating sepsis – and (even more amazingly) that the primary prior art reference teaches treatment of sepsis symptoms:

> ". . . it is noted that **claim 49 is not drawn to a method of treating sepsis**, but rather to a method of administering anti-IFN-g, anti-TNF-a, and anti-IL-6 antibodies to a mammal having a plurality of symptoms of sepsis. Skurkovich teaches administration of the same combination for treatment of autoimmune disorders and AIDS. Because it would be expected[ed] that AIDS would be characterized by fever and organ failure, **Skurkovich teaches treatment of patients with a plurality of symptoms of sepsis**, and thus seeks to solve the same problem as set [forth in] claims 49-50." (emphasis added)

Applicants submit that this argument is outrageous. First, a word search of Skurkovich for the term "sepsis" reveals it is not contained in the document. Second, the plain language of Claim 49 requires not merely "symptoms" but specific symptoms; Claim 49 requires that the symptom of "arterial hypotension" be present, along with one other symptom selected from the group consisting of metabolic acidosis, fever, decreased systemic vascular resistance, tachypnea, and organ failure. Third, a word search of Skurkovich for the term "hypotension" reveals it is not

contained in the document. Fourth, the examiner cites NOTHING for the proposition that "it would be expected that AIDS would be characterized by fever and organ failure." A word search of Skurkovich for "organ failure" reveals it is lacking in the document. Moreover, even if a symptom like "fever" is shared between two different diseases, this says nothing about whether the same treatment of one disease will work for the other disease. In short, this argument is nonsense.

Moreover, Claims 52 and 54 require that the mammal HAVE sepsis. Thus, the Examiner's convoluted argument could not possible apply to Claims 52 and 54. Similarly, Claims 55 and 57 require that the mammal HAVE septic shock. Consequently, the Examiner's argument again has no applicability. And yet, Skurkovich is cited against all of these claims. Clearly, the Examiner's failure to articulate a basis for why Skurkovich is NOT non-analogous art – as to at least these claims – forces a ruling that that Applicants argument is unrebutted.

C. The Examiner Overstates What Skurkovich Teaches

The Board is also asked to take a considered view of what Skurkovich actually teaches. When first introducing Skurkovich (see the Office Action mailed September 2006), the Examiner argued that Applicants' three antibody composition was taught in Claims 3 and 9 of Skurkovich. Looking carefully at Claims 3 and 9 of Skurkovich reveals that the precise three (3) antibody combination is not explicitly taught. Claim 3 teaches two or more antibodies, with the precise three (3) antibody combination being only a possibility – indeed, a possibility that is not called out. Claim 9 is a four (4) antibody combination. Given the rejection is an obviousness rejection, where is the teaching in Skurkovich for the particular three (3) antibody combination presently included in Applicants' claims? Clearly, the Examiner overstates what is taught.

D. The Examiner Ignores Skepticism And Teaching Away By Experts

In 1999, Applicant introduced the Opal et al. reference[1] to show that there is sepsis literature which teaches away from combinations: "Combination anticytokine therapy may exacerbate systemic infection and worsen [the] outcome in experimental sepsis." (see Response filed in October of 1999, Tab A, Abstract, last line). Under the law, skepticism by experts is an objective indicator that has been identified by courts following *Graham*. *See Environmental*

[1] This reference is included in the Evidence Appendix for the convenience of the Board.

Designs, Ltd. v. Union Oil Co. of Cal., 713 F.2d 693, 697-98 (Fed. Cir. 1983) (considering skepticism or disbelief before the invention as an indicator of non-obviousness). Moreover, this skepticism expressed by Opal et al. was based on data that showed a combination therapy that was "uniformly fatal." (see ABSTRACT). Thus, it amounts to teaching away.

In the most recent Final Office Action, the Examiner argues that this is unpersuasive since Opal does not discuss the precise three (3) antibody combination currently claimed. Again, this argument is outrageous. Opal et al. found a particular combination therapy "uniformly fatal" and warned about "combination anticytokine therapy" generally. While this does not mean ALL combination therapy will be uniformly fatal, it shows the lack of predictability – which is the hallmark of non-obviousness. Furthermore, this is not simply a matter of combination therapy not working well; it is a situation where an adverse result, death, has taken place. One skilled in the art would want to avoid this adverse result.

E. The Examiner has provided only conclusory statements

Skurkovich is non-analogous art and there is no rational basis for combining it. The Examiner has offered only the conclusory statement that "the disclosures of Starnes and Doherty would provide one of ordinary skill in the art with the motivation to administer the composition taught by Skurkovich." Why? Back in 1999,[2] when the Starnes publication was cited by Examiner Hamud, he admitted that "Starnes et al do **not** disclose a composition comprising **both** antibodies to TNF-α and IL-6 or a method of treatment with such composition." (Office Action, mailed 07/02/99, p. 3, emphasis added). Similarly, the Examiner noted that Doherty did not administer combinations, but used "**either** anti-IFN- polyclonal antibodies or anti-TNF-polycloncal antibodies . . ." (p. 3-4, emphasis added). In view of these facts, why don't the disclosure of Starnes and Doherty simply motivate one to use single antibodies independently? Certainly, Skurkovich contributes nothing to the question of what would be effective treatment for sepsis. Thus, one skilled in the art looking to solve the sepsis problem sees only single antibody solutions from Starnes and Doherty. The Examiner has no basis for jumping from the single antibody solutions of Starnes and Doherty to a three antibody combination. The Board is reminded that the Supreme Court demands a rational basis for combining art, not merely conclusory statements. In other words, a specific showing by the Examiner is required:

[2] Applicant protests the return to prior art that was cited in 1999 and then removed. This is unfair and costly.

> Often, it will be necessary ... to look to interrelated teachings of multiple patents ... in order to determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue. To facilitate review, this analysis should be made explicit. See, *In re Kahn*, 441 F.3d 977, 988 (CA Fed. 2006) ("[R]ejections on obviousness grounds **cannot be sustained by mere conclusory statements**; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness").

KSR v. Teleflex, Slip Op No. 04-1350 (April 30, 2007). It is not enough to find a three antibody combination out of the sepsis context and simply state that one skilled in the art trying to solve sepsis would somehow put it in context. Thus, a *prima facie* obviousness position has not been established. Moreover, as discussed below, the actual DATA in Doherty and Starnes teaches away.

F. Doherty Administered *Prior* To Challenge Using *Single* Antibodies

The Board is asked to take careful note of the Doherty reference. Doherty uses an experimental system wherein antibody (e.g. a single antibody) is administered six (6) hours before the challenge with LPS. This is NOT reflective of the reality of sepsis, nor meaningful when one considers that ALL of Applicants' claims require administration AFTER symptoms or AFTER sepsis/shock has started. Applicants' unexpected results of Table 5 (discussed more below) are obtained AFTER the animals were challenged.

G. Starnes Administered *Prior* To Challenge Using *Single* Antibodies

The other secondary reference, Starnes, is no better. Like the Doherty group, Starnes et al. also use an experimental system wherein antibody is administered BEFORE the challenge (in this case between 0.5 hours and 2 hours before the challenge). Again, this is not meaningful in view of the fact that the present claims specify treatment AFTER symptoms.

H. Starnes Only Simultaneous Administration Failed

Importantly, looking at Starnes very carefully, one finds (at page 4188, right hand column, first full paragraph, last sentence) that the group attempted an experiment wherein the challenge and the anti-cytokine antibody were given simultaneously:

". . . the simultaneous administration of anti-IL-6 mAb abd TBF-alpha did not result in a significant reduction in mortality [while] pretreating mice with neutralizing anti-IL-6 mAb up until 0.5 h before TNF did significantly reduce TNF-alpha induced mortality."

In short, this shows that the single antibody experiments would not work in the "rescue" mode shown in Applicants' Table 5 results (discussed more below). In such a mode, the challenge is given FIRST, and the antibodies must rescue the animal.

I. Applicants Presented Unexpected Results

In the previous responses, Applicants pointed to the data in Table 5 of the present specification: "The Applicant achieved an unexpected result, however, and overcame the morbidity by administering the combination of anti-TNF antibodies, anti-IFN-γ antibodies and anti-IL-6 antibodies, as shown in Table 5." The data in Table 5 was generated by administering the challenge FIRST and attempting to rescue the animal by administering the antibodies SECOND (which is more reflective of clinical treatment). Applicants asked the Examiner "to take note of the *degree of improved results* obtained with the combination (e.g. 100% survival in Table 5 in a "rescue from lethality" experiment)." Applicant pointed to the *Adams* case (cited by the Supreme Court in the *KSR* decision) in support of non-obviousness, arguing that the unexpected results rebutted any *prima facie* obviousness (without agreeing that the Examiner had even established *prima facie* obviousness).

In response, the Examiner failed to address the *Adams* case. Moreover, the Examiner fails to address the question of "degree." Instead, the Examiner questions the data and speculates – without a basis – about possible results of other experiments (discussed more below).

With respect to questioning the data, the Examiner previously stated that "One of skill in the art would not know whether the results presented in Table 5 are truly unexpected, or are merely differences due to the timing of antibody administration after LPS challenge." In response, Applicants provided a 132 Declaration of Dr. Stafford[3] (at paragraph 5) who points out that that with respect to the data in Table 5: "These differences are NOT due to the timing of administration, since the timing was the same (i.e. 5 minutes post-challenge)."

With respect to speculating about other experiments, the Examiner asked whether "administration of anti-IFN-γ, or combined anti-IFN-γ and anti-TNF-α antibodies at 5 minutes

[3] This is also included in the Evidence Appendix for the convenience of the Board.

post-challenge offer the same degree of protection as the combination of all three antibodies at 5 minutes post challenge." In response, Dr. Stafford (at paragraph 6 of the 132 Declaration) who discusses the percent survival numbers in Table 5 and concludes: "there is nothing in Table 5 to suggest that single antibodies or two antibody combinations could achieve 100% survival." In making this conclusion, Dr. Stafford emphasizes the "degree" of success and failure (something the Examiner appears to overlook). It is not a question of getting some benefit with the claimed three antibody combination; it is a question of getting such excellent results (100% survival) in the face of poor results with other single antibody and double antibody preparations: "the 100% result is quite unexpected in view of the rather poor results obtained with single antibody and two antibody combinations." (see Declaration, paragraph 6).

J. The Examiner Acknowledged The 132 Declaration But Did Not Address All Of The Evidence Therein

Applicants submit that the Examiner is not free to ignore this evidence of unexpected results, which has now been clarified by one skilled in the art. The Examiner has been provided unexpected results within the confines of Table 5. While there are other results in the specification, Applicant believes the data in Table 5 is quite sufficient to establish the point.

Nonetheless, in the most recent Final Office Action (at page 4), the Examiner once again dismisses the unexpected results because Table 5 allegedly "does not present data showing the other possible combinations, namely anti-IL-6 and anti-TNF-alpha, and anti-IFN-g and anti-TNF-alpha." Thus, the Examiner, while acknowledging the existence of the 132 Declaration, simply pushes aside Dr. Stafford's analysis of the data. Importantly, the Examiner ignores the last paragraph of the 132 Declaration, which points to the "rigor" of the animal model. In other words, the Examiner has ignored the fact that the data of Table 5 was generated in a rescue mode – which is the most extreme test. Simply put, Dr. Stafford's point (i.e. that even the three antibody combination's benefits are diminished as one delays the rescue period) is lost on the Examiner. Had the Examiner considered this point, there would be no question about what might or might not be achieved with mere two antibody combinations. Indeed, had the Examiner considered this point of the 132 Declaration, together with the failure data of Starnes (when the challenge and antibody were done simultaneously), the Examiner would see that the methodology of Starnes and Doherty have no applicability to the claimed subject matter.

K. The Examiner's Arguments Are Arbitrary And Based On Unfounded Speculation

Failure to accept the Table 5 data as unexpected results is arbitrary and improper, in view of the total record. First and foremost, one of the two antibody combinations that the Examiner asks for – namely an anti-IL-6 and anti-TNF-alpha combination – is, in fact, included in Table 5. The results indicated (pg 16, lines 7-10) that this particular two-antibody combination did not provide protection:

> "In addition, either anti-TNF alone at 5 and 15 minute post challenge or combination therapies of anti-IL-6/Gamma IFN (5 minute post) or anti-TNF/anti-IL-6 (5 and 15 minute post) could not significantly protect the animals." (emphasis added).

Thus, two different two-antibody combinations in Table 5 are directly compared. In view of this, there is absolutely no basis for speculating that some other two antibody combination would provide the 100% results shown for the now claimed three (3) antibody combination.

Moreover, the single antibody data for anti-TNF completely refutes the Examiner's argument based on Doherty and Starnes. While these researchers may have found "benefit" with an anti-TNF antibody where the antibody is administered PRIOR to the challenge, the Table 5 data of the present specification shows that anti-TNF antibody alone, when administered AFTER the challenge, provides NO benefit. The more rigorous test shows the single antibody "benefits" to be minimal. This confirms the results of the single "simultaneous" experiment by Starnes (discussed above). Since the more rigorous test shows the lack of reliability of the Doherty and Starnes testing approach.

L. CONCLUSION

Appellants submit that, with due consideration to all these factors discussed above, the patentability of the claims is evident. The Appellants submit that the Examiner has not made a *prima facie* case of obviousness. In addition to the fact that the primary prior art reference is non-analogous, it has been misconstrued. Furthermore, the Examiner has not given due weight to the affirmative evidence of non-obviousness. For the foregoing reasons, it is submitted that the examiner's rejections of claims were erroneous, and reversal of these rejections is respectfully requested.

Respectfully submitted,

Dated: June 30, 2008

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CLAIM APPENDIX

1-48. (Cancelled)

49. (Previously Presented) A method of treatment, comprising:

a) providing

i) a mammal having a plurality of symptoms of sepsis, wherein said symptoms comprise arterial hypotension and at least one selected from the group consisting of metabolic acidosis, fever, decreased systemic vascular resistance, tachypnea, and organ failure,

ii) a therapeutic preparation, comprising anti-TNF-alpha, anti-IL-6, and anti-IFN antibodies; and

b) administering said preparation to said mammal wherein said symptoms are reduced.

50. (Previously Presented) The method of Claim 49, wherein said antibodies are polyclonal.

51. (Presently Amended) A therapeutic composition for use with a mammal having a plurality of symptoms of sepsis, wherein said symptoms comprise arterial hypotension and at least one selected from the group consisting of metabolic acidosis, fever, decreased systemic vascular resistance, tachypnea, and organ failure, said therapeutic composition comprising avian anti-TNF-alpha, anti-IL-6, and anti-IFN antibodies.

52. (Previously Presented) A method of treatment, comprising:

a) providing:

i) a mammal having sepsis,

ii) a therapeutic preparation, comprising anti-TNF-alpha, anti-IL-6, and anti-IFN antibodies; and

b) administering said preparation to said mammal wherein said sepsis is reduced.

53. (Previously Presented) The method of Claim 52, wherein said antibodies are polyclonal.

54. (Presently Amended) A therapeutic composition for use with a mammal having sepsis, said therapeutic composition comprising avian anti-TNF-alpha, anti-IL-6, and anti-IFN antibodies.

55. (Previously Presented) A method of treatment, comprising:

a) providing:

i) a mammal having septic shock,

ii) a therapeutic preparation, comprising anti-TNF-alpha, anti-IL-6, and anti-IFN antibodies; and

b) administering said preparation to said mammal wherein said septic shock is reduced.

56. (Previously Presented) The method of Claim 55, wherein said antibodies are polyclonal.

57. (Presently Amended) A therapeutic composition for use with a mammal having septic shock, said therapeutic composition comprising avian anti-TNF-alpha, anti-IL-6, and anti-IFN antibodies.

EVIDENCE APPPENDIX

(132 Declaration – which is of record)

(Opal *et al.* – of record)

PATENT
Attorney Docket No. **OPHD-03282**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: John A. Kink
Serial No.: 09/095,536
Filed: 06/10/98
Entitled: **Prevention and Treatment of Sepsis**

Group No.: 1646
Examiner: Hissong

DECLARATION OF DR. DOUGLAS STAFFORD

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8(a)(1)(i)(A)

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being deposited with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: October 17, 2007

By: Traci E. Light

I, Dr. Douglas Stafford, under penalty of perjury, state that:

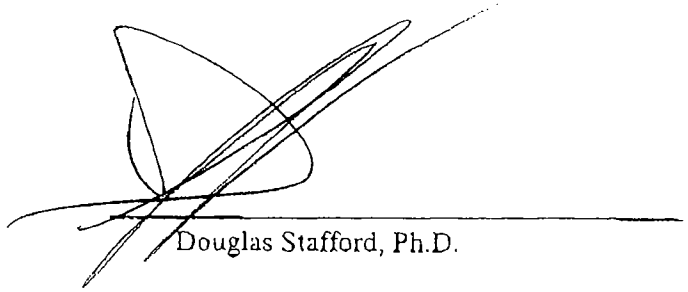
1. I am the former President and CEO of the former assignee of the above-captioned United States Patent Application. I am familiar with the experimental data set forth in the specification. I have graduate degrees in microbiology and immunology.
2. I understand that, in a recent Office Action, the Examiner states that "One of skill in the art would not know whether the results presented in Table 5 are truly unexpected, or are merely differences due to the timing of antibody administration after LPS challenge."
3. I understand that the Examiner also asks whether "administration of anti-IFN-γ, or combined anti-IFN-γ and anti-TNF-α antibodies at 5 minutes post-challenge offer the same degree of protection as the combination of all three antibodies at 5 minutes post challenge."
4. It is important to emphasize that Table 5 shows the results from a particularly aggressive gram-negative sepsis model wherein a lethal dose of LPS is administered and followed by antibody administration (note the language in Example 5: "This model is also considered very aggressive, and the onset of septic shock is quite rapid.") The approach does NOT involve pre-mixing or pre-treatment.

5. Table 5 shows that anti-TNF-α antibodies alone (whether at 5 minutes or 15 minutes post-challenge) are not protective. Table 5 also shows that anti-IFN-γ antibodies in combination with anti-IL-6 antibodies are not protective at 5 minutes post-challenge. By contrast, the combination of anti-IL-6, anti-IFN-γ and anti-TNF-α antibodies provide complete (100%) protection at 5 minutes post-challenge. These differences are NOT due to the timing of administration, since the timing was the same (i.e. 5 minutes post-challenge).

6. Looking strictly at the data in Table 5, it is clear that the degree of results obtained with the three antibody combination, namely 100% rescue, is in sharp contrast to the results obtained with anti-TNF-α alone (where 0% was observed). Similarly, anti-IFN-γ antibodies in combination with anti-IL-6 antibodies achieved only 33% survival. Thus, there is no basis for speculating that "anti-IFN-γ, or combined anti-IFN-γ and anti-TNF-α antibodies at 5 minutes post-challenge offer the same degree of protection as the combination . . ." While such single antibodies or two antibody combinations may provide SOME protection (e.g. 33% to 50% survival), there is nothing in Table 5 to suggest that single antibodies or two antibody combinations could achieve 100% survival. Indeed, the 100% result is quite unexpected in view of the rather poor results obtained with single antibody and two antibody combinations.

7. The rigor of the test is evident from the data in Table 5. More specifically, Table 5 does show that the benefit of the three antibody combination is diminished if the timing of the administration is delayed. This underscores the point that the onset of shock is rapid.

Dated: October 16, 2007



Douglas Stafford, Ph.D.

Potential Hazards of Combination Immunotherapy in the Treatment of Experimental Septic Shock

Steven M. Opal, Alan S. Cross, Jhung W. Jhung, Lynnette D. Young, John E. Palardy, Nicholas A. Parejo, and Curtis Donsky

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Using an actual infection model of *Pseudomonas aeruginosa* sepsis in neutropenic rats, the potential utility of a combination anticytokine approach for the treatment of sepsis was tested. A dimeric tumor necrosis factor binding protein (TNF-BP) consisting of two soluble recombinant human TNF type 1 receptors linked with polyethylene glycol was used with recombinant human interleukin-1 receptor antagonist (IL-1ra). Despite having levels of bacteremia and endotoxemia similar to the control group (survivors, 0/18), 30% of IL-1ra–treated animals survived ($P < .05$); 31% of TNF-BP–treated animals survived ($P < .01$). Unexpectedly, the combination of IL-1ra plus TNF-BP proved to be uniformly fatal (survivors, 0/20). Endotoxin ($P < .0001$) and bacteremia ($P < .01$) levels were >10-fold higher than levels in animals treated with IL-1ra alone, TNF-BP alone, or placebo. Disseminated microabscesses in major organs were found in animals treated with combination immunotherapy. Combination anticytokine therapy may exacerbate systemic infection and worsen outcome in experimental sepsis.

Despite advances in antimicrobial therapy and supportive care of the critically injured patient, septic shock continues to result in an unacceptably high mortality rate that has not changed appreciably in the last three decades [1, 2]. Compelling experimental evidence and considerable clinical evidence now implicate the proinflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF) as major components of the systemic inflammatory process that culminates in septic shock [3]. Numerous laboratory studies in animal models demonstrate that inhibition of TNF [4–6] or IL-1 [7–9] protects against the deleterious effects of excessive cytokine activity and significantly improves the survival rate in experimental septic shock. Recent multicenter clinical trials with anti-TNF monoclonal antibodies [10, 11] and inhibitors of IL-1 activity [12, 13] suggest a modest survival benefit in some patient subgroups; however, the overall benefit of these treatment strategies has been rather disappointing [14, 15].

TNF and IL-1 are pleiotropic molecules that have remarkably redundant physiologic activities of potential relevance to the generation of septic shock [3]. There is evidence that these two cytokines may act synergistically in the generation of systemic inflammatory responses, hypotension, metabolic acidosis, and lethality in experimental sepsis models [16]. Recent evidence suggests that the combination of immunotherapeutic agents directed against different components of the septic process might offer greater protection than would single inhibitors of inflammatory mediators [17].

It has been speculated that the partial protection afforded by inhibitors of either IL-1 or TNF alone might be potentiated if inhibitors of both cytokines were given simultaneously. A recent report [18] clearly demonstrated a survival advantage when endotoxemic rodents are given a combination of inhibitors for IL-1 and TNF simultaneously.

In this current investigation, using an actual infection model of *Pseudomonas aeruginosa* sepsis, we did a series of experiments to determine the potential therapeutic efficacy of combination immunotherapy directed against both major proinflammatory cytokines in experimental septic shock. The neutropenic rat model provides an opportunity to study this combined anticytokine treatment strategy in a host that develops an endogenously mediated, progressive, systemic infection with a virulent strain of *P. aeruginosa*. The model is designed to mimic the sequence of events that may follow the administration of cytoreductive chemotherapy followed by invasive infection with gram-negative bacilli [17].

Materials and Methods

Chemicals and reagents. Chemicals were purchased from Sigma (St. Louis) except for cefamandole (Eli Lilly, Indianapolis). The IL-1 inhibitor used in these experiments was human recombinant IL-1 receptor antagonist (IL-1ra; gift from Synergen, Boulder, CO), a 17-kDa protein that specifically binds to type I and type II IL-1 receptors yet lacks agonist activity and sterically inhibits binding of IL-1α or IL-1β [7, 8]. The TNF inhibitor is a dimeric construct that consists of two recombinant soluble human TNF receptor type I molecules covalently linked to a 20-kDa polyethyl-

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The experimental design of the animal model was reviewed and approved by the university animal care committee.

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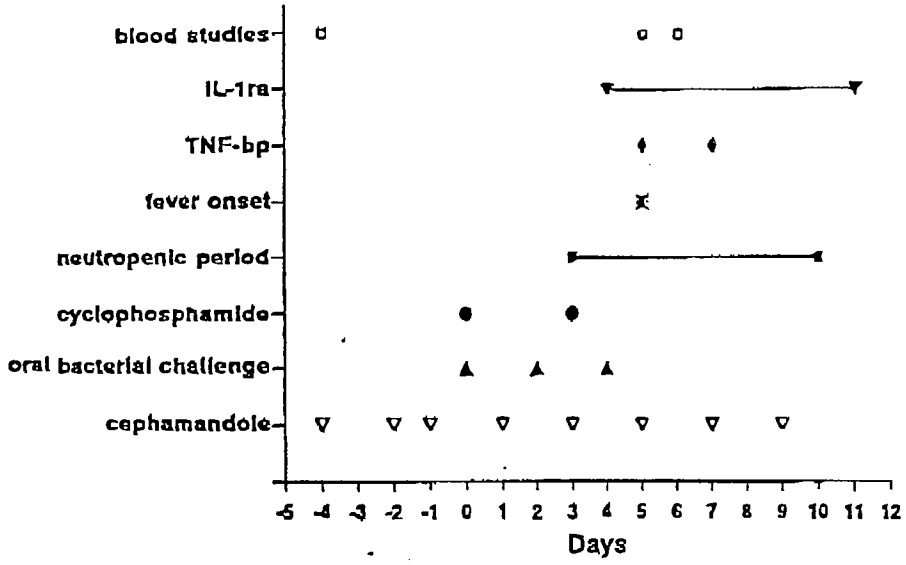

Figure 1. Experimental design of neutropenic rat model of *Pseudomonas aeruginosa* sepsis. IL-1ra, recombinant human interleukin-1 receptor antagonist; TNF-bp, tumor necrosis factor binding protein.

ene glycol moiety (Synergen). This dimeric TNF binding protein (TNF-BP) is a potent inhibitor of TNF activity [18]. An irrelevant IgM monoclonal antibody (B55; Xoma, Berkeley, CA) was given as a control for the TNF-BP; saline was infused as a control for the IL-1ra infusion.

Animal model. Details of this neutropenic rat model have been described [17]. The challenge organism used in these experiments was *P. aeruginosa* 12.4.4 (Fisher-Devlin-Gnabasik immunotype 6), a serum-resistant, virulent, human blood isolate (provided by A. McManus, US Army Institute for Surgical Research, Fort Sam Houston, San Antonio, TX). The organism was stored in whole sheep blood agar (Adams Scientific, West Warwick, RI) at −70°C until use. The day before oral challenge, the isolate was inoculated into trypticase soy broth (Becton Dickinson, Cockeysville, MD) and incubated in an orbital shaker at 37°C. The next day, bacteria were suspended in sterile normal saline and adjusted spectrophotometrically to 10^6 cfu/mL.

The experimental design of the animal model is depicted in figure 1. IL-1ra was administered by continuous infusion using subcutaneous miniosmotic pumps (2mL-1 pumps; Alza, Palo Alto, CA). Two pumps were surgically implanted, and each was loaded with 2 mL of IL-1ra at 100 mg/mL. Each delivered 10 μL/h for 7 days.

Preliminary experiments demonstrated that this method of administration resulted in a continuous therapeutic blood level of IL-1ra at 12.4 ± 4.6 μg/mL over the 7-day infusion (measured by IL-1ra–capture ELISA; Smith CG, Synergen). Endogenous IL-1ra levels in saline-treated control animals were <5 ng/mL. Animals that received saline via miniosmotic pumps served as a control for animals that received TNF-BP alone and for the control group that received the intravenous control monoclonal antibody. The pumps were surgically implanted on day 4 to ensure that therapeutic blood levels would be present throughout the duration of fever and bacteremia.

TNF-BP was given intravenously at a dose of 4.5 mg/kg on the basis of preliminary experiments indicating that this dose should be effective [18]. TNF-BP was administered intravenously via rat tail vein at the onset of fever (day 5) and again 48 h later.

Blood and necropsy studies. Animals were anesthetized with light CO_2. Blood samples for determining amounts of bacterial culture, TNF levels, and endotoxin levels were obtained from the retroorbital plexus at baseline, onset of fever, and 24 h after the onset of fever. Animals were examined twice daily and deaths were recorded. After the death of each lethally infected animal, samples of lung, liver, spleen, kidney, and adrenal tissues were examined histopathologically. Each animal that survived the duration of the study was sacrificed and had similar histopathologic examination. Histologic sections were reviewed by a pathologist who was unaware of the treatment administered to each animal. The lung tissue of each lethally infected animal was extracted and blotted dry; the tissue was then weighed and desiccated at 50°C for 72 h, when dry weights were determined.

Quantitative blood cultures were done by serial dilution in normal saline followed by plating on MacConkey's agar and incubating for 24 h at 37°C. Oxidase-positive, non–lactose-fermenting isolates were further characterized on pseudomonas isolation agar (Difco, Detroit) and serotyped using a polyvalent *P. aeruginosa* antiserum kit (Difco).

Circulating levels of bacterial endotoxin were measured by a quantitative, turbidimetric, amebocyte lysate assay (Cape Cod Associates, Woods Hole, MA) [19]. The endotoxin standard was *Escherichia coli* 0113, which was prepared in both endotoxin-free serum and endotoxin-free water. TNF-α levels were determined using an L929 cytotoxicity assay [20].

Statistical analysis. Results are expressed as mean ± SD; differences were considered significant at $P < .05$. Differences between multiple groups were compared using the Kruskal-Wallis one-way analysis of variance test followed by Dunn's multiple comparisons tests.

Results

Survival. Animals were uniformly febrile within 5 days of the initiation of cyclophosphamide treatment. Control animals

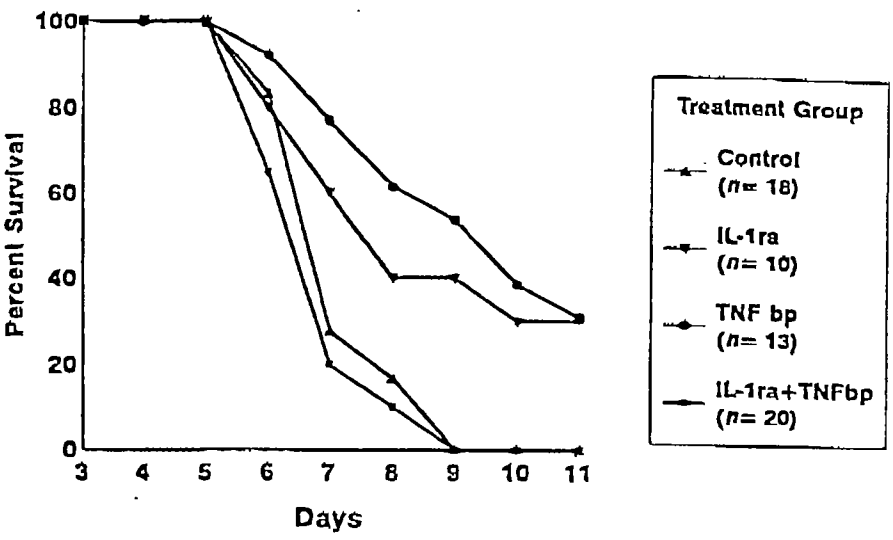

Figure 2. Survival analysis of each group of animals over 12-day experimental period of *Pseudomonas aeruginosa* sepsis. IL-1ra, interleukin-1 receptor antagonist; TNF-bp, TNF-binding protein. IL-1ra + TNF-bp vs. IL-1ra alone or TNF-bp alone, $P < .05$.

($n = 18$) became overtly ill (inactivity, poor appetite, piloerection, and conjunctivitis) and did not survive beyond 72 h of illness. Animals given single-component therapy with either IL-1ra ($n = 10$) or TNF-BP ($n = 13$) had a significant survival advantage compared with the control group ($P < .05$). The combination therapy group, which received both TNF-BP and IL-1ra ($n = 20$), had a rapid and uniformly fatal course that was significantly worse than the course of rats given either immunotherapeutic agent alone ($P < .05$; figure 2.)

Effects of combination immunotherapy on endotoxin, quantitative bacteriology, and TNF levels. Mean endotoxin levels at the onset of fever were 0.66–1.07 ng/mL and did not significantly differ between the treatment groups. At 24 h after the onset of fever, endotoxin levels rose moderately in the control group and in the groups receiving IL-1ra alone or TNF-BP alone. The mean endotoxin levels in these 3 groups now were 1.46–3.4 ng/mL ($P = .07$). However, combination therapy with IL-1ra and TNF-BP resulted in strikingly elevated endotoxin levels that were 10-fold greater than those in any other group ($P < .0001$; figure 3.)

The results of the quantitative blood culture analysis are similar to those from the endotoxin analyses. Quantitative bacteriology revealed low-grade bacteremia with the challenge organism, *P. aeruginosa* 12.4.4., of 34 ± 20 cfu/mL of blood at the onset of fever. The level of bacteremia did not differ significantly in any of the treatment groups. At 24 h later, the mean level of bacteremia had increased to 52–151 cfu/mL in the control group and single component groups treated with either IL-1ra or TNF-BP (not significant). The combination treatment group had strikingly elevated blood culture measurements of 2670 ± 2530 cfu/mL ($P < .01$; figure 4.)

TNF levels did not differ significantly between treatment groups at baseline or at the onset of fever (data not shown). Plasma TNF levels 24 h after the first dose of TNF-BP or in the combination immunotherapy group were <2 pg/mL. TNF levels in the control group were (32.2 ± 5.3 pg/mL); IL-1ra–treated animals had similarly elevated TNF levels (29.9 ± 5.3 pg/mL). TNF levels in these groups were both significantly higher ($P < .05$) than in the TNF-BP–treated group or combination treatment group.

Pathology. Histologic examination of lung, liver, adrenal, and splenic tissues in lethally infected animals from the control group or the groups that received TNF-BP alone or IL-1ra alone showed similar findings. There was evidence of diffuse interstitial edema and pulmonary congestion and uniform evidence of acute tubular necrosis. Focal areas of adrenal hemorrhage were also noted in 59% of autopsy specimens. The histologic findings were indistinguishable between control-treated animals, TNF-BP–treated animals, and IL-1ra–treated animals that succumbed during the 12-day experiment (figure 5A). Animals that survived the experimental phase had evidence of mild interstitial edema but had normal renal tubular anatomy.

In contrast, animals treated with a combination of IL-1ra and TNF-BP had diffuse microabscesses throughout the lung tissue (figure 5B), with focal abscesses in kidney, splenic, and hepatic tissues. These abscesses yielded a pure culture of *P. aeruginosa* 12.4.4.

Lung water determinations, as estimated by wet-to-dry lung ratios, revealed significantly higher levels in the control-treated animals compared with animals who received either anticytokine treatment. The wet-to-dry ratio was 6.24 ± 0.30 for controls, 5.25 ± 0.27 for TNF-BP–treated animals, 4.96 ± 0.40 for IL-1ra–treated animals, and 5.29 ± 5.2 for animals treated with IL-1ra plus TNF-BP ($P < .05$).

Discussion

These results demonstrate the potential risks inherent to simultaneous inhibition of both major proinflammatory cytokines during systemic bacterial infection. The cytokine networks are

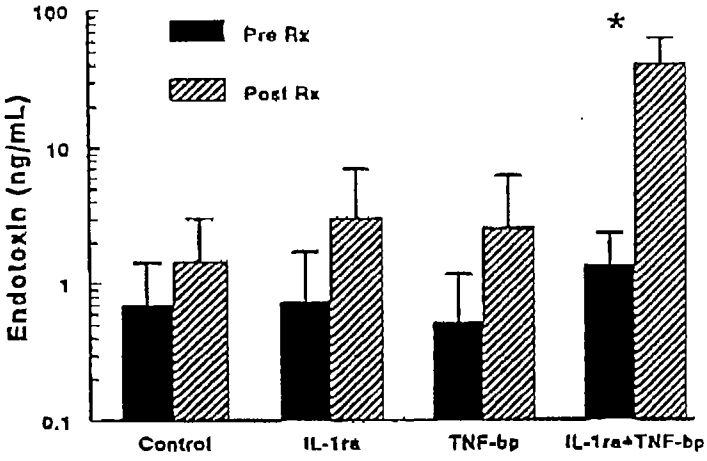

Figure 3. Mean (±SD) endotoxin levels from each group of animals with *Pseudomonas aeruginosa* sepsis at onset of fever (day 5) and 24 h after administration of immunotherapeutic agents (day 6). IL-1ra, interleukin-1 receptor antagonist; TNF-bp, tumor necrosis factor binding protein; Rx, treatment. * IL-1ra + TNF-bp vs. other groups after treatment, $P < .001$.

highly complex and interdependent, with amplification pathways and counter-regulatory mechanisms [3]. Attempts to augment or inhibit one or more of these inflammatory mediators may have unanticipated results in the presence of active infection.

Previous reports indicate that under certain experimental conditions, inhibition of either TNF or IL-1 activity can adversely affect the host response to invasive bacterial infection [21, 22]. TNF inhibition has been shown to exacerbate *Listeria* infection [23], bacterial peritonitis [24], *Salmonella* infection [25], and *Legionella* infection [26] in experimental models. IL-1 inhibition worsens the outcome in certain animal models following bacterial challenge with either *Klebsiella pneumoniae* [27] or *Listeria* species [28].

In the current set of experiments, inhibition of either IL-1 or TNF activity in neutropenic rats did not significantly affect the clearance of bacteremia or endotoxemia. However, combination anticytokine therapy with simultaneous inhibition of both TNF and IL-1 markedly accelerated systemic bacterial invasion, resulting in high-grade endotoxemia, disseminated microabscess formation, and uniform lethality.

These results contrast with a recent report by Russell et al. [18], in which IL-1ra and TNF-BP were shown to provide an additive survival advantage in lipopolysaccharide (LPS)–challenged rats. These authors showed that IL-1ra plus TNF-BP protected animals against renal and metabolic dysfunction and lethality following endotoxin challenge. Another study, however, reported no benefit from combined inhibition of IL-1 and TNF [29]. These apparently conflicting results may be explained by the dichotomous roles of IL-1 and TNF in the host response to invasive bacterial infection. Excessive TNF and IL-1 levels act synergistically to induce an exaggerated systemic inflammatory response to infection that may lead to septic shock and death [2, 21]. However, both IL-1 and TNF also act in concert to activate host defenses in response to invading microbial pathogens, which protects the host from bacterial infection.

C3H/HeJ mice are known to be highly resistant to endotoxin challenge because of a transcriptional and translational block in LPS-induced TNF synthesis [30]. While these mice are endotoxin-hyporesponsive, they are highly susceptible to lethal infection with virulent strains of *E. coli*. The admin-

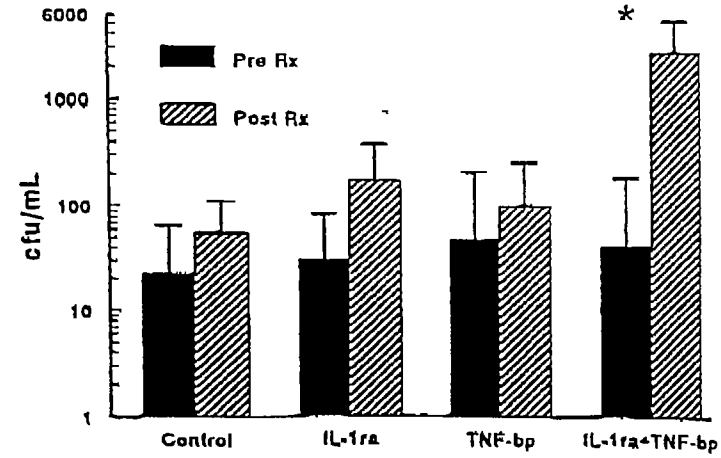

Figure 4. Colony counts (mean ± SD) of *Pseudomonas aeruginosa* 12.4.4 from blood cultures from each group of animals at onset of fever (day 5) and 24 h after administration of first dose of immunotherapy (day 6). IL-1ra, interleukin 1 receptor antagonist; TNF-bp, tumor necrosis factor binding protein; Rx, treatment. * IL-1ra + TNF-bp vs. other groups after treatment, $P < .01$.

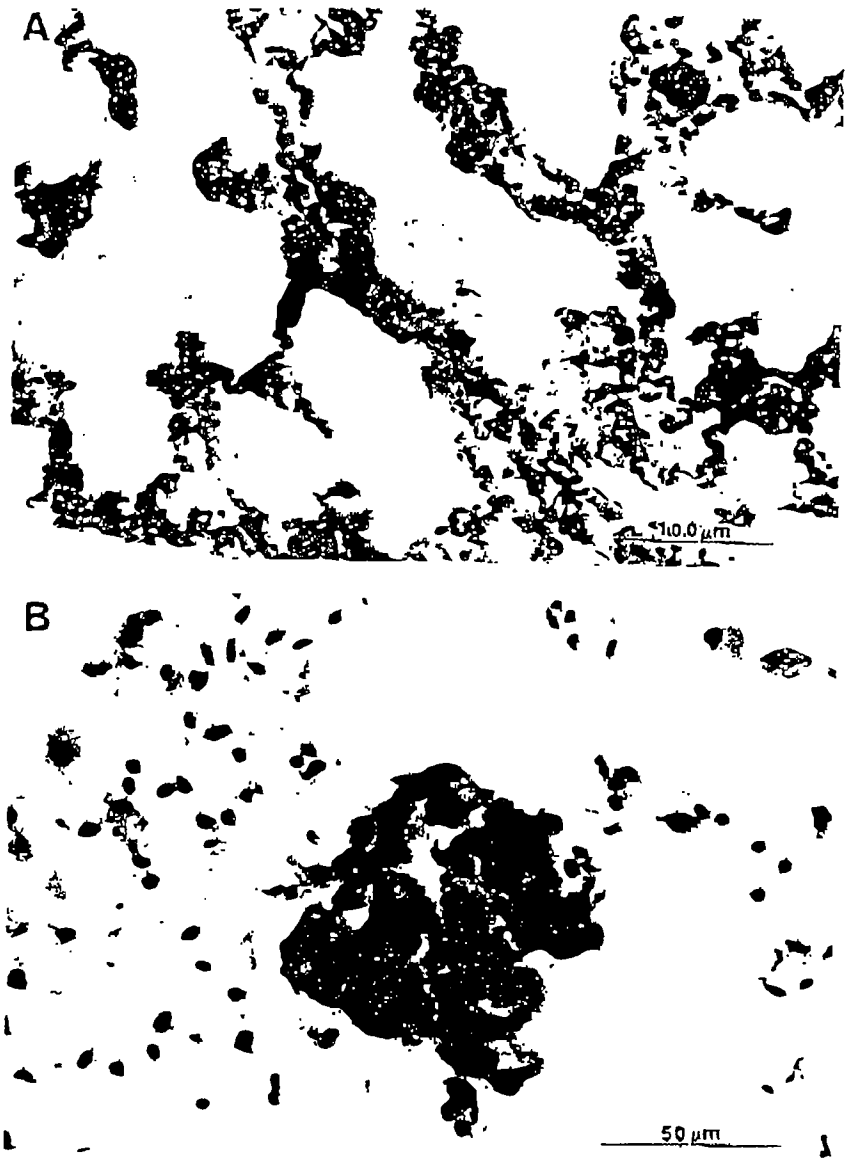

Figure 5. Histopathologic samples of lung tissue from lethally infected animals with *Pseudomonas aeruginosa* sepsis. A, interstitial edema and pulmonary congestion in control group (magnification, ×288); B, microabscess of challenge organism *P. aeruginosa* 1244 in lung tissue of animal treated with combination of interleukin-1 receptor antagonist and tumor necrosis factor binding protein (magnification, ×720).

istration of small amounts of both recombinant IL-1 and TNF restores host defenses of C3H HeJ mice to levels comparable with those of LPS-responsive strains of mice (e.g., C3H/HeN) [31]. A doubling of the amount of IL-1 administered resulted in loss of its protective capacity. Therefore, cytokine-mediated host defenses may be important in protecting the host from systemic infection. Since small changes in the dose of exogenously administered cytokines abolish this protective effect, it appears that the cytokine response is very tightly regulated. Invasive bacterial pathogens that can induce sepsis may behave like classic intracellular pathogens (i.e., *Salmonella*, *Listeria*, or *Legionella* species). Coordinated activation of the phagocytic system is required for effective killing of these pathogens. Since TNF and IL-1 are important activators of mononuclear phagocytes, inhibition of both cytokines simultaneously may induce a state of unrestrained growth of the bacterial pathogens, resulting in overwhelming sepsis [32].

Animal models that use an intoxication challenge of LPS or a large intravenous bolus of bacterial organisms exhibit the deleterious manifestations of proinflammatory cytokine activation in sepsis models. While these models provide valuable information regarding the pathophysiology of endotoxemic shock, they do not provide an opportunity to examine the host-pathogen interactions seen in an actual, invasive, bacterial infection. Inhibitors of the inflammatory mediators with anticytokines might prove to be beneficial in intoxication models yet be detrimental in infection models [33]. Similar dichotomous results have been observed with monoclonal antibodies directed against the β_2 integrins on the neutrophil surface [34].

Inhibition of either one of the cytokines in the neutropenic rat model of *Pseudomonas* sepsis results in a survival benefit for treated animals. There appears to be sufficient redundancy in the physiologic roles of either of the major proinflammatory cytokines to maintain the host response to invasive bacterial infection. However, simultaneous inhibition of both cytokines may not allow an adequate host response to bacterial invasion, resulting in overwhelming infection and lethality.

It is important to recognize that this experimental model uses profoundly neutropenic animals and that specific antimicrobial therapy against the invasive strain of *P. aeruginosa* was not used. It is possible that the results would differ if an adequate neutrophil response was available in non-neutropenic animals. Moreover, the simultaneous administration of antimicrobial therapy against the invasive strain of *P. aeruginosa* may have avoided the detrimental effects of combination anticytokine therapy in these septic animals. These findings show that it will be essential to adequately control the infectious component of the septic process to avoid potential toxicity from combination immunotherapy in future clinical trials.

In conclusion, inhibition of either IL-1 or TNF in immunocompromised animals with *Pseudomonas* infection results in a survival benefit while the simultaneous inhibition of both cytokines results in exacerbation of the invasive infection and a rapidly lethal outcome. The inhibition of one major proinflammatory cytokine may attenuate the systemic inflammatory response while the host defenses are maintained through activation of the uninhibited inflammatory cytokine.

These results emphasize the importance of animal models that utilize an actual infectious challenge with bacteria that can actively replicate and disseminate within the host in the study of septic shock. Before new experimental agents in septic shock can be used in humans, infection models must be studied to assure that inhibition of inflammatory mediators will not adversely impair the host response to infection. Endotoxin challenge models and other bacterial intoxication models may not provide a satisfactory system by which to test the potential deleterious effects of innovative agents in the treatment of septic shock. Further investigations are necessary to determine the most efficient means to maintain the appropriate host response to microbial invasion while limiting the detrimental effects of systemic activation of inflammatory mediators in septic shock.

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References

1. Centers for Disease Control. Increase in national hospital discharge survey rates for septicemia—United States: 1979–1987. MMWR 1990;39:31–4.
2. Bone RC. The pathogenesis of sepsis. Ann Intern Med 1991;115:457–69.
3. Dinarello CA. The proinflammatory cytokines interleukin-1 and tumor necrosis factor and treatment of the septic shock syndrome. J Infect Dis 1991;63:1177–84.
4. Tracey KJ, Fong Y, Hesse DG, et al. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteremia. Nature 1987;330:662–4.
5. Beutler B, Milsark IW, Cerami AC. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. Science 1985;229:868–72.
6. Hinshaw LB, Tekamp-Olson P, Chang AC, et al. Survival of primates in LD_{100} septic shock following therapy with antibody to tumor necrosis factor (TNF alpha). Circ Shock 1990;30:279–92.
7. Ohlsson K, Bjork P, Bergenfeldt M, Hageman R, Thompson RC. An interleukin-1 receptor antagonist reduces mortality from endotoxin shock. Nature 1990;348:550–2.
8. Fischer E, Marano MA, Van Zee KJ, et al. Interleukin-1 receptor blockade improves survival in hemodynamic performance in *E. coli* septic shock, but fails to alter host responses to sublethal endotoxemia. J Clin Invest 1992;89:1551–7.
9. Wakabayashi G, Gelfand JE, Burke JF, Thompson RC, Dinarello CA. A specific receptor antagonist for interleukin-1 prevents *Escherichia coli*–induced shock in rabbits. FASEB J 1991;5:338–43.
10. Fisher CJ Jr, Opal SM, Dhainaut JF, et al. Influence of an anti-tumor necrosis factor monoclonal antibody on cytokine levels in patients with sepsis. Crit Care Med 1993;21:318–27.
11. Abraham E, Wunderlink R, Silverman H, et al. Efficacy and safety of monoclonal antibody to human tumor necrosis factor-alpha in patients with sepsis syndrome. JAMA 1995;273:934–41.
12. Fisher CJ Jr, Slotman GJ, Opal SM, et al. Initial evaluation of human recombinant interleukin-1 receptor antagonist in the treatment of sepsis syndrome: a randomized, open-labelled, placebo-controlled trial. Crit Care Med 1994;22:12–21.
13. Fisher CJ Jr, Dhainaut JF, Opal SM, et al. Recombinant human interleukin-1 receptor antagonist in the treatment of patients with sepsis syndrome, results from a randomized, double-blind, placebo-controlled trial. JAMA 1994;271:1863–93.
14. Abraham E, Raffin TA. Sepsis therapy trials: continued disappointment or reason for hope? JAMA 1994;271:1876–8.
15. Eidelman LA, Sprung CL. Why have new effective therapies for sepsis not been developed? Crit Care Med 1994;20:1330–4.
16. Waage A, Espervik T. Interleukin-1 potentiates the lethal effect of tumor necrosis factor-alpha/cachectin in mice. J Exp Med 198867:1987–92.
17. Cross AS, Opal SM, Palardy JE, Bodmer MW, Sadoff JC. The efficacy of combination immunotherapy in experimental *Pseudomonas* sepsis. J Infect Dis 1993;167:112–8.
18. Russel DA, Tucker KK, Khinookoswong N, Thompson RC, Cohno T. Combined inhibition of interleukin-1 and tumor necrosis factor in rodent endotoxemia: improved survival in organ function. J Infect Dis 1995;171:1528–38.
19. Novitsky TJ, Roslansky PF, Siber GR, Warren HS. Turbidimetric method for quantifying serum inhibition of limulus amoebocyte lysate. J Clin Microbiol 1985;20:211–6.
20. Opal SM, Cross AS, Kelly NM, et al. Efficacy of a monoclonal antibody directed against tumor necrosis factor in protecting neutropenic rats from lethal infection with *Pseudomonas aeruginosa*. J Infect Dis 1990;161:1148–52.
21. Dinarello CA, Gelfand JA, Wolff SM. Anticytokine strategies in the treatment of the systemic inflammatory response syndrome. JAMA 1993;269:1829–35.
22. Havell EA. Evidence that TNF has an important role in antibacterial resistance. J Immunol 1989;143:2894–9.
23. Nakane A, Minagawa T, Kato K. Endogenous tumor necrosis factor (cachectin) is essential to host resistance against *Listeria monocytogenes* infection. Infect Immun 1988;56:2563–8.

24. Echtenacher B, Falk W, Mannel DN, et al. Requirement for endogenous tumor necrosis factor/cachectin for recovery from experimental peritonitis. J Immunol 1990;145:3762–6.
25. Tite JP, Dougan G, Chatfield SN. The involvement of tumor necrosis factor in immunity to *Salmonella* infections. J Immunol 1991;147:3161–4.
26. Blanchard DK, Djeu JY, Klein TW, et al. Protective effects of tumor necrosis factor in experimental *Legionella pneumophila* infections of mice via activation of PMN function. J Leukoc Biol 1988;43:429–35.
27. Mancilla J, Garcia P, Dinarello CA. Interleukin-1 receptor antagonist can either reduce or enhance the lethality of *Klebsiella pneumoniae* sepsis in newborn rats. Infect Immun 1993;61:926–32.
28. Havell EA, Moldawer LL, Helfgott D, Kilian PL, Sehgal PB. Type I interleukin-1 receptor blockade exacerbates murine listeriosis. J Immunol 1992;148:1486–92.
29. McNamara MJ, Norton JA, Nauta RJ, Alexander HR. Interleukin-1 receptor antibody (IL-1rab) protection and treatment against lethal endotoxemia in mice. J Surg Res 1993;54:316–21.
30. Beutler B, Krochin N, Milsark IW, et al. Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. Science 1986;232:977–80.
31. Cross AS, Sadoff JC, Kelly N, Bernton E, Gemski P. Pretreatment with recombinant murine tumor necrosis factor-alpha/cachectin and murine interleukin-1 alpha protects mice from lethal bacterial infection. J Exp Med 1989;169:2021–7.
32. Cross AS, Asher L, Seguin M, et al. The importance of a lipopolysaccharide-initiated, cytokine-mediated host defense mechanism in mice against extra-intestinally invasive *Escherichia coli*. J Clin Invest 1995; 96:676–86.
33. Cross AS, Opal SM, Sadoff JC, Gemski P. Choice of bacteria in animal models of sepsis. Infect Immun 1993;61:2741–7
34. Eichacker PQ, Hoffman WD, Farese A, et al. Leukocyte CD18 monoclonal antibody worsens endotoxemia and cardiovascular injury in canines with septic shock. J Appl Physiol 1993;74:1885–92.

RELATED PROCEEDINGS APPENDIX

(No attachments are required for this Brief)